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THE OCCURRENCE OF MYCOPLASMAS IN SELECTED WILD NORTH AMERICAN WATERFOWL

D. R. Goldberg,[1] M. D. Samuel,[1] C. B. Thomas,[2] P. Sharp,[2] G. L. Krapu,[3] J. R. Robb,[4] K. P. Kenow,[5] C. E. Korschgen,[5] W. H. Chipley,[6] M. J. Conroy,[6] and S. H. Kleven[7]

[1] National Biological Service, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA
[2] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA
[3] National Biological Service, Northern Prairie Science Center, 8711 37th Street SE, Jamestown, North Dakota 58401, USA
[4] Ohio Cooperative Fish and Wildlife Research Unit, Ohio State University, 1735 Neil Avenue, Columbus, Ohio 43210, USA
[5] National Biological Service, Upper Mississippi Science Center, P.O. Box 818, La Crosse, Wisconsin 54602, USA
[6] Georgia Cooperative Fish and Wildlife Research Unit, School of Forest Resources, University of Georgia, Athens, Georgia 30602, USA
[7] Department of Avian Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA

ABSTRACT: We determined the prevalence of mycoplasma infection in breeding mallard (*Anas platyrhynchos*) and canvasback (*Aythya valisineria*) hens and their broods from the central United States (1988 to 1990); and wintering American black duck (*Anas rubripes*) and mallard hens from the eastern United States (1990 to 1993). Mycoplasmas were isolated by culturing tracheal swabs from 656 live birds and tissue samples from 112 dead waterfowl. Nine (18%) of 51 mycoplasma isolates were identified as *Mycoplasma anatis; M. anatis* was recovered from four mallards, a black duck, and a gadwall (*Anas strepera*) duckling. Nineteen (37%) of 51 mycoplasma isolates were identified as *Mycoplasma cloacale;* these isolates were obtained from mallard, canvasback, and black duck adults, and from a mallard duckling. Additional unspeciated mycoplasmas were isolated from mallards, black ducks, and one canvasback.

Key words: Wild ducks, mallard, *Anas platyrhynchos*, canvasback, *Aythya valisineria*, American black duck, *Anas rubripes*, mycoplasmas, *Mycoplasma anatis*, *Mycoplasma cloacale*, gadwall, *Anas strepera*, survey.

INTRODUCTION

Mycoplasmas frequently have been isolated from domestic chickens (*Gallus gallus*) and turkeys (*Meleagris gallapavo*) (Kleven, 1994). Many mycoplasmas, particularly *M. gallisepticum* and *M. synoviae*, cause clinical disease affecting primarily respiratory and reproductive systems, and synovia of joints (Yoder, 1991). In commercial poultry, infection with pathogenic mycoplasmas leads to economic losses through declines in laying and hatching rates, and through poor growth (Stipkovits, 1979). Decreased egg production and hatchability also have been demonstrated in captive-reared wild turkeys experimentally infected with *Mycoplasma gallisepticum* (Rocke et al., 1988).

Less is known about the occurrence and effects of mycoplasmas in ducks. The earliest reported mycoplasma obtained from a domestic duck was isolated during a 1952 epizootic in Canada (Walker and Bannister, 1953). Since that time, mycoplasmas have been recovered from domestic or semi-domestic ducks, primarily in Europe. *Mycoplasma anatis* appears to be the most commonly isolated and pathogenic of the species reported in ducks. *Mycoplasma anatis* and *M. gallisepticum* were isolated in England from domestic ducks with respiratory disease and other abnormalities (Jordan and Amin, 1980). *Mycoplasma anatis* was associated with an epizootic affecting the central nervous system of domestic ducks in Hungary (Ivanics et al., 1988), and was recovered from wild northern shovelers (*Anas clypeata*) in Spain (Poveda et al., 1990). In Egypt, *M. anatis* and other mycoplasmas were recovered from Pekin ducks (*Anas platyrhynchos*) and eggs, although no disease was observed (El-Ebeedy et al., 1987). In addition, *Mycoplasma cloacale* has been found in healthy wild ducks in France (Bradbury et al., 1987) and healthy semi-domestic ducks in

Yugoslavia (Bencina et al., 1987). There are no published reports, however, of mycoplasma isolates from wild ducks in North America.

As part of our studies to assess the health status of wild waterfowl in the central and eastern United States, we obtained samples for mycoplasma isolation from mallards (*Anas platyrhynchos*), canvasbacks (*Aythya valisineria*), and American black ducks (*Anas rubripes*). Our objectives for this portion of the study were to determine the prevalence of mycoplasmas in these free-living ducks.

MATERIALS AND METHODS

We collected samples for mycoplasma isolation from breeding mallards and canvasbacks in the central United States and from wintering mallards and black ducks in the eastern United States (Fig. 1). We obtained tracheal swabs from breeding mallards in the prairie pothole region, near Jamestown, North Dakota (USA) (46°25′N, 98°55′W), and near Hitterdal, Minnesota (USA) (47°00′N, 96°15′W) during the breeding seasons of 1988, 1989, and 1990. These mallards were trapped on small ponds early in the breeding season using decoy traps with spring-door openings (Sharp and Lokemoen, 1987). A game-farm mallard hen was placed in a central cylinder in each trap to attract wild mallard pairs into the outer compartments. Tracheal swab samples were obtained from canvasbacks during the summer of 1990 at Agassiz National Wildlife Refuge (NWR), near Thief River Falls, Minnesota (48°18′N, 96°00′W). Canvasbacks were captured in decoy traps (Anderson et al., 1980) and in large swim-in traps (Haramis et al., 1982).

Nests of breeding mallard hens were monitored closely during the expected time of hatch, and ducklings were captured before they left the site (<1 day old). Additional nests of mallards inside a predator exclosure (Greenwood et al., 1990) were located with nest dragging methods (Higgins et al., 1969). Eggs from canvasbacks were collected from the nest, artificially incubated, and ducklings were returned to the nest shortly after hatch (C. E. Korschgen, unpubl.). Tracheal swab samples for mycoplasma isolation were collected from mallard and canvasback ducklings.

In the fall and winter of 1991 to 1992 and 1992 to 1993, tracheal swabs were obtained from juvenile mallard and black duck hens at the Ottawa NWR near Oak Harbor, Ohio (USA) (41°37′N, 83°10′W). Juvenile and adult black duck hens also were sampled at the Duck River

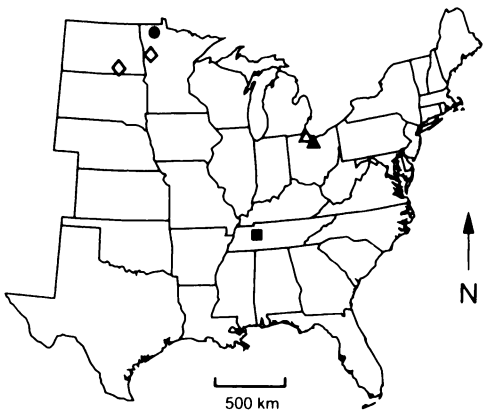

FIGURE 1. Map of mid-continental United States. Study sites were in North Dakota, Minnesota, Tennessee, and Ohio.

Unit of the Tennessee NWR near New Johnsonville, Tennessee (USA) (35°50′N, 87°50′W) in the winters of 1990 to 1991 and 1991 to 1992. Wintering mallards and black ducks were trapped in large permanent swim-in traps and with cannon nets (Addy, 1956; Day et al., 1980).

Mallard and black duck hens at each study site were fitted with backpack type radio transmitters (Dwyer, 1972) or collar radio transmitters in the fall and winter of 1992 to 1993 (Montgomery, 1985). Radio transmitters were implanted in the abdominal cavity of canvasback hens following the methods of Olsen et al. (1992) or implanted subcutaneously on the back (C. E. Korschgen, unpubl.). Short-range radio transmitters were implanted subcutaneously on the back of canvasback ducklings (C. E. Korschgen, unpubl.) and attached to the back of male mallard ducklings (Krapu and Luna, 1991).

Radio-telemetry was used to monitor bird survival and facilitate carcass recovery of radio-marked hens and ducklings previously sampled. In addition, carcasses from radio-marked canvasbacks located in the summers of 1988 and 1989, and black ducks and mallards from the Ohio site which died in the 1990 to 1991 field season also were examined. Carcasses of wild waterfowl without radio transmitters were also collected opportunistically during routine field

activities on the study sites. Birds either were frozen or shipped fresh to the National Wildlife Health Center (NWHC), Madison, Wisconsin (USA) for necropsy and tissue collection. Trachea, ovary, and lung tissues were frozen and saved for later mycoplasma isolation, although only tracheal tissues were taken in 1988. Tissues from birds with marked autolysis were excluded.

A medium prepared by the formulation of Jordan (1983) was used for transport, primary isolation, and passage of mycoplasmas. Briefly, the medium contained PPLO broth base (Difco Laboratories, Detroit, Michigan, USA), porcine serum (Sigma Chemical Company, St. Louis, Missouri, USA), yeast extract (Difco), glucose (Difco), arginine hydrochloride (Sigma), thallium acetate (Sigma), nicotinamide adenine dinucleotide (Sigma), penicillin (Sigma), and phenol red (Difco) as a pH indicator. The medium was used in three forms: liquid broth as described, agar plates made by the addition of 1% molten agarose (Difco), and a biphasic transport medium in which an agar slant was overlayed with broth.

Tracheal swabs taken for mycoplasma isolation were immersed in the biphasic transport medium, and the swab discarded. These samples were frozen and shipped to NWHC for analysis. Frozen swab samples later were thawed and incubated at 37 C for 1 wk, or until evidence of growth of fermentative microorganisms. Tubes with apparent growth were checked for bacterial contamination by passage of 0.1 ml sample into 5.0 ml brain heart infusion (BHI) broth (Difco). If no growth occurred in either mycoplasma broth or BHI, 0.5 ml of the liquid portion of the sample was transferred into 5.0 ml of fresh mycoplasma broth and incubated again for 1 wk. Contaminated cultures were filtered (1 ml) through a 0.45 μm pore-diameter cellulose acetate filter (Corning Glass Works, Corning, New York, USA), to remove extraneous bacteria before passage into mycoplasma broth. After 1 wk, or when growth was evident, samples were overlaid onto mycoplasma agar and incubated at 37 C with 5% CO_2 with a pan of distilled water placed in the bottom of the incubator for added humidity. Periodically, plates were examined microscopically (4×) for mycoplasma growth, and samples were considered negative if no colonies were seen on the agar after 1 mo. Tissue sections were finely minced under aseptic conditions, diluted in mycoplasma broth (approximately 5 g tissue in 25 ml broth), and then treated in the same manner as the swab samples.

A culture of *M. anatis* was obtained from American Type Culture Collection (ATCC No. 25524, Rockville, Maryland, USA) and grown and passed in tubes of mycoplasma medium, with rabbit serum (Sigma) substituted for porcine serum. After 48 hr incubation at 37 C, the cells were collected by centrifugation at 17,000 × *G* for 30 min, and washed three times with phosphate buffered saline. The protein concentration was determined by the method of Lowry et al. (1951), and the antigen was diluted to 1 mg protein per ml in mycoplasma broth.

Two 3-mo-old New Zealand white rabbits (*Oryctolagus cuniculus*) were used for immunization to the *M. anatis* antigen, following the protocol of Senterfit (1983), with the following modifications. The initial injection and all blood collections were carried out under anesthesia with Innovar-Vet (Pitman-Moore, Washington Crossing, New Jersey, USA) at a dose of 0.1 ml/kg given intramuscularly (IM). Rabbits were aroused with an equal intravenous dose of Naloxone hydrochloride (LyphoMed, Inc., Melrose Park, Illinois, USA).

The initial mycoplasma inoculation consisted of 1.2 ml of antigen emulsified in an equal volume of Freund's complete adjuvant (Sigma), and 0.1 ml administered into 24 subcutaneous sites on the back of each rabbit. The rabbits were given a booster every 3 wk with 0.5 ml injections of the antigen, given IM into four sites. The rabbits were bled periodically, and the sera collected by centrifugation at 1,500 × *G* for 20 min. Antibody titers were checked by an enzyme-linked immunosorbent assay (ELISA) procedure (Thomas and Sharp, 1988), using our stock *M. anatis* as the antigen. The specificity of the antisera was verified by the ELISA test. There was minimal cross-reactivity with antigens from stock *M. gallisepticum*, *M. synoviae*, *M. gallinarum*, *M. gallipavonis*, *M. iners*, and *M. iowae*.

Thirty-four isolates were examined using sodium dodecyl sufate-polyacrylamide gel electrophoresis (SDS-PAGE) (Thomas and Sharp, 1988). All isolates then were tested in an immunobinding procedure (Kotani and McGarrity, 1985) using rabbit antisera specific for eight common species of avian *Mycoplasma* (*M. gallisepticum*, *M. meleagridis*, *M. synoviae*, *M. anatis*, *M. gallinaceum*, *M. gallipavonis*, *M. iners*, and *M. iowae*) and one *Acholeplasma* spp. (*A. laidlawii*). The antisera used, except for *M. anatis*, were obtained from supplies previously prepared by Thomas and Sharp (1988) and Sharp et al. (1991). Homologous antigen, serum deletion, and conjugate deletion controls were included in each assay. Identifications made using the immunobinding assay were confirmed by Western blot analysis (Thomas et al., 1991).

Twenty viable isolates not identified by immunobinding, were tested with a larger panel of antisera using a direct immunofluorescence assay (DIA) (Talkington and Kleven, 1983).

These isolates were chosen because they were viable upon subculture and therefore appropriate for use with the DIA. In addition to the eight mycoplasma species listed above for the immunobinding assay, these isolates were tested with antisera to *M. gallinarum*, *M. pullorum*, *M. glycophilum*, *M. lipofaciens*, *M. cloacale*, *M. columbinum*, and *M. columborale*. However, not all isolates were tested against all of these antisera. The remaining non-viable, unidentified isolates then were tested by immunobinding assay using antiserum to *M. cloacale* obtained from Dr. J. G. Tully at the National Institute of Health (Fredrick, Maryland). Methods for *M. cloacale* antisera production were according to Senterfit (1983), with the exception of the use of the Ribi adjuvant system (Ribi ImmunoChem Research, Inc., Hamilton, Montana, USA) as the delivery vehicle (Tully, 1993).

TABLE 1. Mycoplasma isolates recovered from tissues and tracheal swabs of breeding mallards and canvasbacks from North Dakota and Minnesota (1988 to 1990), mallards and black ducks from Ohio (1990 to 1991 to 1992 to 1993), and black ducks from Tennessee (1990 to 1991 and 1991 to 1992).

Source	Total samples	Number of *Mycoplasma* spp. isolates recovered: *M. cloacale*	*M. anatis*	Species unknown	No mycoplasma recovered
Trachea	109	0	4	2	103
Lung	91	5[a]	3	6[a]	78
Ovary	26	1	1	4[b]	21
Tracheal swab	656	13[a]	1	11[a,b]	633

[a] One sample contained both a *Mycoplasma cloacale* and a *Mycoplasma* spp. isolate.
[b] One sample contained two *Mycoplasma* spp. isolates.

RESULTS

We recovered *Mycoplasma* spp. from five (19%) of 26 ovaries, 13 (14%) of 91 lungs, and six (5.5%) of 109 tracheas from the carcasses sampled (Table 1). We also isolated mycoplasmas from 23 (3.5%) of 656 swab samples from live birds examined. *Mycoplasma anatis* and *Mycoplasma cloacale* were the only species identified in our cultures. *Mycoplasma anatis* was isolated from all tissue types, nonpreferentially, and from one tracheal swab sample; it comprised nine (18%) of 51 mycoplasma isolates (Table 1). *Mycoplasma cloacale* was isolated from tracheal swabs, lungs, and one ovary (Table 1) and represented 19 (37%) of the mycoplasmas recovered. Unspeciated mycoplasma isolates included both glucose fermenters and nonfermenters, and were recovered most frequently from ovary and lung tissues. In addition, we recovered more than one species of mycoplasma from five birds. Thirty-four of the isolates were examined using SDS-PAGE. Six different electrophoretic banding patterns were observed; the samples were grouped according to these six profiles. One group of isolates subsequently was identified as *M. anatis* and a second group as *M. cloacale*. Based on the observed banding patterns, twelve unspeciated isolates were distributed among the remaining four groups, thus several mycoplasma species probably were present.

Mycoplasma anatis was isolated from the tissues of four (3.6%) of 112 carcasses (Table 2): two mallard hens that died near Jamestown in the summers of 1988 and 1989, a male mallard that died in a decoy trap near Jamestown in 1989, and a mallard hen found near Hitterdal in 1989. *Mycoplasma anatis* was not isolated from the carcasses of any breeding canvasbacks or wintering mallards or black ducks. *Mycoplasma anatis* was recovered from one (0.2%) of 656 tracheal swab samples, a black duck captured in Tennessee. Incidental to our study of breeding mallard hens and broods, we isolated *M. anatis* from two of 49 tracheal swabs from mallard males and from the tracheal swab of a 1-day-old gadwall (*Anas strepera*) duckling, sampled in North Dakota.

Mycoplasma cloacale was isolated from the tissues of six (5.3%) of 112 birds necropsied (Table 2). It was recovered from the lungs of adult male and female mallards found near Jamestown in the summer of 1989. We isolated *M. cloacale* from the tissues of juvenile ducks from Ohio: one mallard that died during the 1990 to 1991 field season, and from a mallard carcass and a black duck carcass found during the 1992 to 1993 field season. We obtained *M.*

TABLE 2. Prevalence of mycoplasmas from breeding ducks in the central United States and wintering ducks in the eastern United States, 1988 to 1993.

State/duck species Sample type	Total samples	*Mycoplasma anatis*	*Mycoplasma cloacale*	Species unknown
Minnesota/Mallard				
Swab-adult[a]	107	0 (0)[b]	2 (1.9)	1 (0.9)
Swab-duckling	54	0 (0)	1 (1.8)	0 (0)
Carcass-adult	10	1 (10)	0 (0)	0 (0)
Carcass-duckling	5	0 (0)	0 (0)	0 (0)
North Dakota/Mallard				
Swab-adult[a]	102	0 (0)	0 (0)	0 (0)
Swab-duckling	89	0 (0)	2 (2.2)	0 (0)
Carcass-adult	24	3 (13)	2 (8.3)	5 (21)
Carcass-duckling	18	0 (0)	0 (0)	0 (0)
Minnesota/Canvasback				
Swab-adult[a]	19	0 (0)	1 (5.3)	0 (0)
Swab-duckling	59	0 (0)	0 (0)	0 (0)
Carcass-adult	1	0 (0)	0 (0)	0 (0)
Carcass-duckling	44	0 (0)	0 (0)	1 (2.3)
Ohio/Mallard				
Swab-adult[a]	80	0 (0)	2 (2.5)	2 (2.5)
Carcass-juvenile	3	0 (0)	2 (67)	1 (33)
Ohio/Black duck				
Swab-adult[a]	79	0 (0)	3 (3.8)	3 (3.8)
Carcass-juvenile	6	0 (0)	1 (17)	1 (17)
Tennessee/Black duck				
Swab-adult[a]	67	1 (1.5)	2 (3.0)	3 (4.5)
Carcass-adult	1	0 (0)	1 (100)	0 (0)

[a] Only females sampled.
[b] Number positive (percent positive).

cloacale from the ovary of the only adult black duck necropsied from the Tennessee study. *Mycoplasma cloacale* was isolated from 13 (2.0%) of 656 tracheal swabs taken, from mallard, canvasback, and black duck hens from all sites except North Dakota. No mycoplasma isolates were recovered from tracheal swabs of mallard ducklings in 1988, two *M. cloacale* isolates were recovered in 1989 (from the same brood), and one *M. cloacale* isolate was recovered in 1990. No mycoplasmas were isolated from swabs of canvasback ducklings.

Unspeciated mycoplasmas were recovered from five of 34 adult mallard carcasses collected during the breeding season (Table 2). An unspeciated mycoplasma was isolated from the lung of one of 44 canvasback duckling carcasses, but not from any of the 23 mallard ducklings. One adult canvasback was necropsied; no mycoplasmas were recovered. Unspeciated mycoplasmas also were isolated from juvenile ducks (a mallard and a black duck) from Ohio in 1990 to 1991. One unspeciated mycoplasma was isolated from tracheal swab samples of 430 breeding hens and broods; eight unspeciated mycoplasma cultures were isolated from 226 wintering ducks.

DISCUSSION

There are no previous reports of mycoplasma isolations from wild ducks in North America. We recovered mycoplasmas from 17 (15%) of 112 carcasses necropsied; four (3.6%) of these isolates were *M. anatis* and six (5.4%) were *M. cloacale*.

Few of the carcasses examined had lesions associated with mycoplasma infections. However, esophagitis, tracheitis, and vaginitis, lesions typically associated with mycoplasmosis, were observed grossly in two of the three hens from which *M. anatis* was isolated; one bird died from duck plague, while the second died from severe tracheitis. The role of opportunistic mycoplasma infection in wild birds is unknown, but we speculate that there was some association between active *M. anatis* infections and mortality in these ducks. Wild birds infected with certain species of mycoplasma also may be at higher risk from secondary infections with other pathogens in their environment. Mycoplasmas have synergistic effects with other agents, such as goose parvovirus in goslings (Kisary et al., 1976), and avian influenza virus in ducklings (Roberts, 1964).

Poveda et al. (1990) isolated mycoplasmas from 7 (17%) of 42 oropharynx and tracheal swabs examined in a limited survey of wild European waterbirds. They were unable to recover mycoplasmas from mallards, green-winged teal (*Anas crecca*) and common pochards (*Aythya ferina*), but isolated *M. anatis* from northern shovelers and Eurasian coots (*Fulica atra*). The prevalence of *Mycoplasma* spp. in swab samples from breeding mallards in North Dakota and Minnesota was <5% over 3 yr in our study; the prevalence in adult canvasbacks also was near 5%. We recovered mycoplasmas from 7% of swabs from the birds wintering in Ohio and Tennessee. *Mycoplasma cloacale* was recovered more frequently than *M. anatis* from our samples. Our results may have been an underestimate of the actual prevalence of mycoplasmas, however. Swab sampling techniques often result in false negatives (Clyde and McCormack, 1983), especially in samples frozen for prolonged periods (Samuel et al., 1995), or with high densities of competing bacterial flora (Jordan, 1983). In our study, the prevalence of each mycoplasma type appeared to be higher in tissues than tracheal swabs. These differences may be a result of sampling methodology. In a survey of apparently healthy wild turkeys, Fritz et al. (1992) also found that mycoplasmas were recovered more often from tissue samples (45%) than swabs (25%). It also is possible that these mycoplasmas are not normally present in the trachea, but may preferentially colonize other tissues.

Based on our findings, we believe that *M. cloacale* and *M. anatis* are common species of mycoplasma in wild ducks in North America. Although the pathogenic effects of *M. cloacale* in ducks are undocumented, it has been isolated previously in geese affected by inflammation of the cloaca and phallus (Stipkovits et al., 1986). We observed no association of *M. cloacale* with disease in the ducks we sampled. Isolations of unspeciated mycoplasmas also were fairly common, but we have no information about their effects on waterfowl. We were unable to isolate mycoplasmas usually associated with clinical problems in domestic poultry, particularly *Mycoplasma gallisepticum*, which often has been recovered from domestic ducks (Jordan, 1983). Therefore, we doubt that wild ducks are an important source of infection for domestic poultry operations in the central region of North America.

Mycoplasmas often are transmitted vertically and have caused considerable reduction in productivity in poultry operations (Yoder, 1991). We isolated mycoplasmas from 1-day-old ducklings, providing evidence of vertical transmission in wild birds. *Mycoplasma anatis* is pathogenic to ducklings and eggs, and causes reduced growth rates in young birds (Samuel et al., 1995). Although the effects of *M. anatis* and other mycoplasmas on the productivity of wild duck populations are uncertain, we believe there is a need for further research.

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